IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| Application No. | 10/736,889 | Art Unit: | 1642 |
| Applicant: | Georges *et al.* | Examiner: | Yao, Lei |
| Date Filed: | December 15, 2003 | Conf. No. | 5738 |
| Docket No. | 112418.147 (AUR-013US) | Cust. No. | 23483 |
| Title: | **Vimentin Directed Diagnostics and Therapeutics for Multidrug Resistant Neoplastic Disease** | | |

## DECLARATION OF DR. CORINNE BENQUET PURSUANT TO 37 C.F.R. § 1.132

Dear Sir:

In connection with the above-referenced patent application, I, Corinne Benquet, declare as follows:

1. I received my Ph.D. in 1998 from The University of Montreal and have spent more than 9 years involved in biochemical research. My curriculum vita along with a list of publications, presentations and patents is enclosed hereto as **Attachment A**.

2. I am gainfully employed at Aurelium Biopharma, Inc., the assignee of the above-referenced application, as Section Head, Preclinical Research.

3. I have read the above-referenced patent application.

4. I understand the application to claim a method of diagnosing and treating cancer. In particular, the application describes a method in which the level of cell surface expression of a full-length vimentin protein is measured on both a test neoplastic cell and a control non-neoplastic cell using an anti-vimentin antibody linked to a detectable label. If a level of cell surface-expressed vimentin is present on the test neoplastic cell as compared to the level of cell

surface-expressed vimentin present on the known drug-susceptible control non-resistant, neoplastic cell, then the test neoplastic cell is potentially neoplastic.

5. I also understand that the application claims a method of diagnosing and treating multi-drug resistant cancer. For example, the application describes a method in which the level of cell surface expression of a full-length vimentin protein is measured on both a test neoplastic cell and a control non-resistant, neoplastic cell that is susceptible to a particular drug using an anti-vimentin antibody linked to a detectable label. If an increased level of cell surface-expressed vimentin is present on the test neoplastic cell as compared to the level of cell surface-expressed vimentin present on the known drug-susceptible control non-resistant, neoplastic cell, then the test neoplastic cell is potentially multi-drug resistant.

6. I am familiar with the Office Action dated May 3, 2006 and the Final Office Action dated November 2, 2006, and I have read the references cited in the Office Action and Final Office Action.

7. In these Office Actions, I understand that claims 10, 12, and 14-19 were rejected over the combination of Meschini, Fanger, and Heidenthal. In my opinion, none of the references teach or suggest the detection of cell-surface-expressed vimentin.

8. Meschini does not describe the detection of cell-surface-expressed vimentin. Throughout the text of the article, the authors continually refer to staining *cytoskeletal elements*, which are located within cells and include actin filaments, microtubules, and intermediate filaments (see Meschini, pg. 617-618; Figs. 2c and 2d). Meschini's results demonstrate labeling of the cytoskeletal network. In particular, Figures 2c and 2d clearly show that the fluorescent antibody labeling has occurred inside the cells, where the label stained intermediate filaments containing vimentin (Fig. 2c and 2d). I do not see any discernible labeling on the cell surface.

In my opinion, the authors did not perform the experiments necessary to show cell-surface expression of vimentin. However, Meschini did show that P-glycoprotein ("PgP") was expressed on the surface of neoplastic cells. The results for those experiments are shown in Figure 4. Note the difference in staining between Figures 2 and 4. In Figure 4, the PgP staining is distributed around the periphery of the cells. This is consistent with cell surface staining, which is absent from the cells shown in Figure 2. Therefore, it is my opinion that the only discernible vimentin labeling shown in Meschini is intracellular.

9. Meschini also describes the experimental procedures that were used for vimentin and PgP detection (see Meschini, Materials and Methods, *Flow cytometry*, pg. 616). Specifically, the experimental procedure used for PgP detection is clearly recognized as being associated with the detection of cell surface-associated proteins. (I and other researchers have often used similar experimental procedures to detect cell surface proteins.) This is because the procedure described in Meschini assures that the labeled cells will remain viable, and thus intact, during the labeling procedure.

In contrast, the experimental procedures used in Meschini for detection of vimentin are strikingly different from the PgP detection procedures (see Meschini, Materials and Methods, *Flow cytometry*, pg. 616). Briefly, the authors fixed the cells in 2% paraformaldehyde, and then labeled the cells using fluorescent antibodies mixed in Nonidet P-40, which is a detergent commonly used to permeabilize cell membranes (see Meschini, Materials and Methods, *Flow cytometry*, pg. 616). In my opinion, this procedure would kill the cells, making them more permeable, and thus the vimentin label could detect internal vimentin. This method does not allow for the discernible detection of cell-surface-expressed vimentin.

10. The Final Office Action also refers to a flow cytometry website procedure as showing that researchers in my field would use paraformaldehyde fixation when labeling cell surface antigens (see "Paraformaldehyde Fixation of Cells," Iowa State University ("Iowa State")). In my opinion, the Iowa State reference shows a method of preserving antibody labeling for later

detection, not the fixation of cells prior to labeling. I, and others in my field, do not fix cells prior to labeling cell surface antigens. As stated in Iowa State, "fixed cells have a permeable membrane-the dye would enter all the cells" (see Iowa State, pg. 1). If the label enters all of the cells, it would be nearly impossible to discern any labeling on the cell surface. Thus, Iowa State does not disclose a method of fixing cells prior to antibody labeling because this procedure would not allow discernible detection of cell surface antigens.

11. In my opinion, Fanger only discloses administration of LDL or acetylated-LDL to patients, while Heidenthal describes denatured LDL binding to vimentin *in vitro*. There is no mention of a method for detecting multidrug resistance in a patient by detecting cell-surface expressed vimentin in either reference as teaching or suggesting any of the steps of recited in claims 10, 12, and 14-19.

12. In the Final Office Action, I understand that claims 66, 68, and 70-74 were rejected over the combination of Thomas, Fanger, and Heidenthal. It is my opinion that none of the references describes or suggests the detection of cell-surface-expressed vimentin.

13. Thomas describes the labeling of intermediate filaments throughout the text of the article (see Thomas, pp. 2700-2702). Even in the discussion, Thomas limits the discussion of vimentin expression to the expression observed in intermediate filaments (see Thomas, pg. 2702). This reference does not show the detection of cell-surface-expressed vimentin.

With regard to the figures in Thomas (Figures 1b, 1c, and 3), I do not discern any labeling indicative of cell surface expression of vimentin. Rather, Figures 1 and 3 show labeling throughout the interior of the cell (see Figs. 1 and 3). It is nearly impossible to determine whether any labeling is associated with the cell surface of the cells. It is my opinion that the authors did not perform the experiments necessary to show cell-surface-expressed vimentin.

14. The Final Office Action states that Moisan *et al.* (2006) *J. Leuk. Biol.* 79: 1-10 ("Moisan") shows that cell-surface-expressed vimentin was known to be associated with abnormally growing or stressed cells (see Final Office Action, pg. 5). Moisan does not support the conclusion that vimentin is expressed on the surface of *cancer* cells. Moisan shows cell surface expression of vimentin on neutrophils. It is well known that neutrophils are normal, common cells found in the blood. I do not consider neutrophils to be a model for cancer, and I am not aware of any researchers in my field that consider neutrophils to be a model for cancer. Moisan shows that cell surface expression of vimentin is associated with apoptosis. Apoptosis is a normal cellular process that unfortunately does not typically occur in cancer cells. The article is therefore directed to subject matter that would not interest researchers attempting to identify diagnostic tools for cancer. Thus, because Moisan used cells that were not models for cancer to study apoptosis, a cellular process that is completely unrelated to cancer diagnostics, I would not consider this article to suggest that vimentin on the surface of cancer cells could be useful for cancer diagnostics.

15. My opinion regarding Fanger and Heidenthal is stated above. Neither of these references describes or suggests the detection of cell-surface-expressed vimentin.

16. For all of the reasons presented in paragraphs 11-13, I do not read the Thomas, Fanger, Heidenthal, and Moisan references as describing or suggesting a method for detecting a cancer cell in a patient, as recited in claims 66, 68, and 70-74.

17. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States

Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: April 23, 2007

Corinne Benquet, Ph.D.

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| Applicant: | Georges *et al.* | Art Unit: | 1642 |
| Serial No.: | 10/736,889 | Examiner: | Yao, Lei |
| Filing Date: | December 15, 2003 | Customer No. | 23483 |
| Title: | **Vimentin Directed Diagnostics and Therapeutics for Multidrug Resistant Neoplastic Disease** | Conf. No. | 5738 |

# Attachment A

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## *PROFESSIONAL EXPERIENCE*

**Aurelium BioPharma, Montréal, Qc, Canada**
Biopharmaceutical company developing therapeutics and diagnostic tools for the treatment of sensitive and drug-resistant cancers.

**October 2004-Present** Section Head, Preclinical Research

**Conjuchem Inc., Montréal, Qc.,Canada**
Biopharmaceutical company developing long lasting compounds.

**July 2003- October 2004** Head of Preclinical Pharmacology

Managed the Pharmacology/Biology aspect of preclinical development. Planned and coordinated internal and external pharmacology evaluation (PK/PD) of compounds in Development.

- Designed and conducted scientific studies within a product development regulatory context
- Acted as project leader by setting and implementing project related goals and objectives to ensure project timelines were met.
- Established relevant preclinical models and supportive pharmacokinetic characterization in order to optimize development and maximize therapeutic activity.
- Identified academic collaborators and specialized contract research organizations (CRO) to carry out *in vitro* and/or *in vivo* work and managed all aspect of collaboration, including initial contact, contract, negotiation, protocol design, study monitoring, troubleshooting, final data analyses and reporting.
- Participated in the presentation of study results and development strategy plans at scientific meetings.
- Contributed to Investigator's brochure and regulatory filings (TPD, FDA).
- In collaboration with the other groups within the Development Department, contributed to the preparation of the Clinical Development Plans for the company's products (DAC-GRF).
- Attended and participated in meetings with potential partners and regulatory authorities to present pharmacological data.

**Conjuchem Inc. (continuation)**
**May 2000 – July 2003** Section Leader, Cell Biology

Managed all *in vitro* cell biology and pharmacology studies, both in house and contracted, performed as part of feasibility and/or other projects. Collaborated with academia and industry. Supervised and trained junior scientists and technicians.

- Full responsibility to develop and implement specific cell-based bioassays for *in vitro* assessment of compounds activity and toxicity. Selection of lead compounds for further drug development process.
- Identification of academic collaborators and CRO to carry out *in vitro* and *in vivo* models that cannot be performed in house.
- In charge of the overall project management, study execution, data evaluation, protocol and report generation.
- Communication with peers in other groups as well as with senior management.
- Writing of *in vitro* section of patent applications.
- Involved in projects in different therapeutic areas including: Endocrinololy and Diabetes, Cancer.

## Compatigène Inc., Montreal, Qc, Canada

**Sept 98- May 2000** Research Scientist, Immunology and Cell Biology

Managed cell culture and supervised the establishment of cytotoxic cell lines in order to develop new therapies to increase the compatibility in allogogeneic bone marrow transplantation.

## Guy Bernier Research Center, Maisonneuve-Rosemont Hospital, Montreal, Qc

**1997 - 1998** Research Assistant

- Effect of environmental polluants on the oxydative metabolism of human polymorphonuclear leukocytes.
- Evaluation of cerebrovascular stroke on the immune response.
- Signal transduction pathways associated with the cytotoxic activity of anti-B4 blocked ricin immunotoxins.

## *EDUCATION*

**Ph.D. in Pharmacology**, University of Montreal,Canada
Immunopharmacology laboratory, Guy Bernier Research Center, Maisonneuve-Rosemont Hospital
Supervisor: Edouard Kouassi, PhD

- **Clinical project:** Biological effects of low doses rhGM-CSF in patients after autologous bone marrow transplantation.
- **Research project:** Effects of bacterial endotoxins and inflammatory cytokines on the oxydative metabolism of human polymorphonuclear leukocytes.

**M.Sc. in Biology**, University of Quebec in Montreal (UQAM), Canada
TOXEN Laboratory. Supervisor: Michel Fournier, PhD

- **Project:** Modulation of exercise-induced immunosuppression by dietary polyunsaturated fatty acids in mice.

## *Honors and Awards*

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| 1991-1992 | PAFACC (UQAM) |
| 1993-1994 | Bio-Méga / Boehringer Ingelheim fellowship |
| | U de M - F.E.S – Pharmacologie fellowship |
| 1994-1995 | Bio-Méga / Boehringer Ingelheim fellowship |
| | U de M - F.E.S – Pharmacologie fellowship |
| 1995-1996 | FCAR fellowship |

## *Professional Societies*

American Association of Pharmaceutical Scientists (AAPS)
The Endocrine Society

## *PATENT*

PCT WO 02/062,844: Long lasting growth hormone releasing factor derivatives.
Bridon D., Boudjellab N., Léger R., Robitaille M., Jetté L ., **Benquet C** .

## *PAPERS AND COMMUNICATIONS*

### *ORIGINAL PAPERS*

Jette L, Léger R, Thibaudeau K, **Benquet C**, Robitaille M, Pellerin I, Paradis V, van Wyk P, Pham K, Bridon DP. Human growth hormone-releasing factor (hGRF)1-29-albumin bioconjugates activate the GRF receptor on the anterior pituitary in rats: identification of CJC-1295 as a long-lasting GRF analog. Endocrinology. 2005 Jul;146(7):3052-8.

Roger Léger, **Corinne Benquet**, Xicai Huang, Omar Quraishi, Pieter van Wyk and Dominique Bridon. 2004. Kringle 5 peptide–albumin conjugates with anti-migratory activity. Bioorganic and Medicinal Chemistry Letters. 14(4): 841-845.

Roger Léger, Martin Robitaille, Omar Quraishi, Elizabeth Denholm, **Corinne Benquet**, Julie Carette, Pieter van Wyk, Isabelle Pellerin, Nathalie Bouquet-Gagnon, Jean-Paul Castaigne and Dominique Bridon. 2003. Synthesis and In vitro analysis of atrial natriuretic peptide-albumin conjugates. Bioorganic and Medicinal Chemistry Letters. 13(20): 3569-3573.

Kim, J-G, Baggio, L.L., Bridon, D.P., Castaigne, J-P., Robitaille, M.F., Jetté, L., **Benquet, C** and Drucker, D.J. 2003. Development and characterization of a glucagon-like Peptide 1-albumin conjugate: the ability to activate the glucagon-like Peptide 1 receptor in vivo. Diabetes. 52(3):751-9

**Benquet, C.** et Kouassi, E. 1998. Lipid A-induced oxidative stress requires protein tyrosine kinases and protein kinase C activation, but not intracellular calcium mobilization in human polymorphonuclear leukocytes. Proceedings of the Western Pharmacology Society. 41: 207-210.

Flipo, D., Fournier, M., **Benquet, C.**, Roux, P., Le Boulaire, C., Pinsky, C.,. LaBella, FS and Krzystyniak, K. 1998. Increased apoptosis, changes in intracellular calcium, and functionnal alterations in lymphocytes and macrophages after in vitro exposure to static magnetic field. Journal of Toxicology and Environmental Health. 54: 63-76.

Asselin, P., **Benquet, C.**, Krzystyniak, K., Brousseau, P. et Fournier, M. 1996. In vivo indomethacin reverse exercise-induced immunosuppression in rats. International Journal of Immunopharmacology. 18: 491-497.

**Benquet, C.**, Krzystyniak, K., Savard, R., Guertin, F., Oth, D.et Fournier, M. 1994. Modulation of exercise-induced immunosuppression by dietary polyunsaturated fatty acids in mice. Journal of Toxicology and Environmental Health. 43: 225-237.

### *COMMUNICATIONS*

**Corinne Benquet**, Jean-Paul Castaigne, Khan Pham, Martin Robitaille and Lawrence A. Frohman. CJC-1295 (DAC-GRF), A Long Acting GRF Analog, Enhances Pulsatile GH Secretion, Increases IGF-I Levels, And Restores Linear Growth. The Endocrine Society's 86th Annual Meeting. New Orleans, Louisiana, USA. June 16-19, 2004

**Corinne Benquet**, Isabelle Pellerin, Karen Thibaudeau, Roger Léger, Xicai Huang, Martin Robitaille, Catherine Gagnon, Dominique Bridon and Jean-Paul Castaigne. The DAC Technology Enables Extended Half-Life And Efficacy Of Peptide After *In Vivo* Covalent Binding To Albumin: Applications To DAC-GRF (CJC-1295) and DAC-GLP-1 (CJC-1131). The Endocrine Society's 86th Annual Meeting. New Orleans, Louisiana, USA. June 16-19, 2004.

**Corinne Benquet**, Roger Léger, Xicai Huang, Karen Thibaudeau, Dominique Bridon and Jean-Paul Castaigne. CJC-1131 (DAC:GLP-1) binds covalently *in vivo* to endogenous albumin: update on the DAC technology. American

Diabetes Association-64th Scientific sessions. Orlando, Florida, USA. June 4-8, 2004

M. Robitaille, **C. Benquet**, I. Pellerin, L. Jetté, J. Carette, K.Pham, P.Bakis, N. Arya, C. Beaupré, V. Paradis, D. Calamba, H.K. Nguyen and D. Bridon. Novel long acting GRF analogs with high potencies and extended half-life in rats. 18th American Peptide Symposium. Boston, Massachusetts, USA. July 19-23, 2003

**Corinne Benquet**, Lucie Jetté, Isabelle Pellerin, Manon Lacoursière, Véronique Paradis, Martin Robitaille, Julie Carette, Elizabeth Denholm,and Dominique Bridon. Bioactivity and pharmacokinetic characteristics of novel atrial natriuretic peptide (ANP) analog. *The FASEB Journal.* 17(5): A1084.
Experimental Biology 2003. San Diego, CA, USA. April 11-15,2003.

Robitaille, M., Carette, J., Bakis, P., Arya, N., Sonoc, I., Beaupré, C., L'Archevêque, B., Pham, N., Van Wyk, P., Sekhon, D., Quraishi, O., Thibaudeau, K., Jetté, L., **Benquet, C.**, St-Jean, M., Paradis, V., Bousquet-Gagnon, N., Phan, K., Tremblay, S., Calamba, D., Castaigne, J-P., Bridon, D. Long lasting derivatives of the GLP-1 agonist exibit high potency and extended pharmacokinetics when bioconjugated to albumin in vivo. International peptide symposium. Serono, Italy, August 31st-September 6th 2002.

**Benquet, C.**, Thibaudeau, K., Smith, D.C., Huang, X., Pham, K. Van Wyk, P., Bridon, D. and Castaigne, J.P. New long half-life kringle 5 based DAC™-peptides with potent antimetastic activity. *Proceedings of the American Association for Cancer Research.* 43: 2002.
American Association for Cancer Research 93rd annual meeting congress. San Francisco, CA, USA. April 6-10, 2002

Kouassi, E., **Benquet, C.** et Abdouh, M. Augmentation du flux calcique et altérations morphologiuqes induites par le chlorure de methyl mercure dans les polymorphonucléaires humains. 66ième congrès de l'ACFAS. Université Laval, Québec, 11-15 mai 1998.

Kouassi, E. et **Benquet, C.** Lipid A-induced oxidative stress requires protein tyrosine kinases and protein kinase C activation, but not intracellular calcium mobilization in human polymorphonuclear leukocytes. 41st Annual Meeting of the Western Pharmacology Society. Mazatlan, Mexico, January 25-30 1998.

**Benquet, C.** et Kouassi, E. Rôle des PTK et de la PKC dans la production de peroxyde d'hydrogène induite par le lipide A dans les granulocytes humains. Journée de la recherche Gabriel L. Plaa. 26 Avril 1996. Montréal, Québec, Canada.

**Benquet, C.**, Roy, D.C., Gyger, M. et Kouassi, E. Biological effects of *in vivo* infusion of GM-CSF in bone marrow transplants. *The Canadian Journal of Infectious Diseases.* 6(suppl C): 369C, 1995
19th International Congress of Chemotherapy. July 16-21 1995, Montréal, Québec, Canada.

**Benquet, C.**, Kouassi, E., Roy, D.C. et Gyger, M. Priming of oxygen free radical production by GM-CSF *in vivo* after autologous bone marrow transplantation (ABMT). *Canadian Journal of Physiology and Pharmacology.* 72(suppl 1): 253,1994
XIIth International Congress of Pharmacology. July 24-29 1994, Montréal, Quebec, Canada.

Goulet, A.C., **Benquet,C.** et Kouassi, E. Différenciation de la lignée monoblastique humaine THP-1 par l'acide rétinoïque et le phorbol 12-myristate 13-acétate. Journée de la recherche de l'Hôpital Maisonneuve -Rosemont. Montréal, Québec. 3 juin 1994.

**Benquet, C.**, Beaudry, M. et Kouassi, E. Stimulation de la fonction de production de radicaux libres par le GM-CSF *in vivo* après greffe de moelle osseuse. *Médecine/Sciences.* 9(suppl 1): 54, 1993
35ième Réunion Annuelle du Club De Recherches Cliniques Du Québec. Pointe-Au -Pic, Québec. 30 Septembre-2 Octobre 1993.

**Benquet, C.**, Beaudry, M. et Kouassi, E. Évaluation de la fonction de production de radicaux libres d'oxygènes lors

de l'administration de GM-CSF chez des patients ayant reçu une greffe de moelle osseuse autologue. *Union Médicale du Canada.* Janvier 1994. p.48
Journée de la recherche de l'Hôpital Maisonneuve -Rosemont. Montréal, Québec. 4 juin 1993.

**Benquet, C.**, Guertin, F., Savard, R., Oth, D.et Fournier, M. Corrective effect of the exercice-unduced immunosuppression by W-3 polyunsaturated fatty acids. 8th International Congress of. Budapest, Hongrie. August 24-29, 1992.

**Benquet, C.**, Guertin, F., Savard, R., Oth, D.et Fournier, M. Correction par la nutrition de l'effet immunosuppresseur induit par l'exercice. 60ième congrès de l'ACFAS. Montréal, Québec. 11-15 Mai 1992.

**Benquet, C.**, Guertin, F., Flipo, D., Oth, D et Fournier, M. Effect of W-3 and W-6 polyunsaturated fatty acids on immune parameters. 6th Annual Spring Meeting-Auberge Mont Gabriel Mont Rolland, Quebec, Canada. March 13-16, 1992.

**Benquet, C.**, Guertin, F., Mansour, S., Oth, D. et Fournier, M. Effect of saturated and unsaturated fatty acids on immune parameters. 34th annual symposium of The Society of Toxicology of Canada. Montréal, Québec, Canada. December 5-6, 1991.

**Benquet, C.**, Tremblay, A., Deveaux, M., Savard, R., Fournier, M. Immunity of exercised rodents. Canadian Federation of Biological Socieites- 34th Annual Meeting. Kingston, Ontario, Canada. June 9-11, 1991.

Flipo, D., **Benquet, C.**, Krzystyniak, K.,Fournier, M. A new approach to monitor blastic assay using cytofluorometry. 5th Annual Spring Meeting- Chateau Lake Louise, Alberta, Canada. March 8-12. 1991.

**Benquet, C.**, Tremblay, A., Savard, R., Fournier, M. The effect of exercise on the immune system in rodents. . 5th Annual Spring Meeting -Chateau Lake Louise, Alberta, Canada. March 8-12, 1991.